manufacturer's instructions. The sense strand of the resulting dsRNA for each of the target genes is given in Table 8-AG.

C. Laboratory Trials to Test dsRNA Targets, Using Artificial Diet for Activity Against the Larvae of the House Cricket, *Acheta domesticus*

[0337] House crickets, *Acheta domesticus*, were maintained at Insect Investigations Ltd. (origin: Blades Biological Ltd., Kent, UK). The insects were reared on bran pellets and cabbage leaves. Mixed sex nymphs of equal size and no more than 5 days old were selected for use in the trial. Double-stranded RNA was mixed with a wheat-based pelleted rodent diet (rat and mouse standard diet, B & K Universal Ltd., Grimston, Aldbrough, Hull, UK). The diet, BK001P, contains the following ingredients in descending order by weight: wheat, soya, wheatfeed, barley, pellet binder, rodent 5 vit min, fat blend, dicalcium phosphate, mould carb. The pelleted rodent diet was finely ground and heat-treated in a microwave oven prior to mixing, in order to inactivate any enzyme components. All rodent diet was taken from the same batch in order to ensure consistency. The ground diet and dsRNA were mixed thoroughly and formed into small pellets of equal weight, which were allowed to dry overnight at room temperature.

[0338] Double-stranded RNA samples from targets and gfp control at concentrations 10 μg/μl are applied in the ratio 1 g ground diet plus 1 ml dsRNA solution, thereby resulting in an application rate of 10 mg dsRNA per g pellet. Pellets are replaced weekly. The insects are provided with treated pellets for the first three weeks of the trial. Thereafter untreated pellets are provided. Insects are maintained within lidded plastic containers (9 cm diameter, 4.5 cm deep), ten per container. Each arena contains one treated bait pellet and one water source (damp cotton wool ball), each placed in a separate small weigh boat. The water is replenished ad lib throughout the experiment.

[0339] Assessments are made at twice weekly intervals, with no more than four days between assessments, until all the control insects had either died or moulted to the adult stage (84 days). At each assessment the insects are assessed as live or dead, and examined for abnormalities. From day 46 onwards, once moulting to adult commences, all insects (live and dead) are assessed as nyumph or adult. Surviving insects are weighed on day 55 of the trial. Four replicates are performed for each of the treatments. During the trial the test conditions are 25 to 33° C. and 20 to 25% relative humidity, with a 12:12 hour light:dark photoperiod.

D. Cloning of a MLB Gene Fragment in a Vector Suitable for Bacterial Production of Insect-Active Double-Stranded RNA

[0340] What follows is an example of cloning a DNA fragment corresponding to an MLB gene target in a vector for the expression of double-stranded RNA in a bacterial host, although any vector comprising a T7 promoter or any other promoter for efficient transcription in bacteria, may be used (reference to WO0001846).

[0341] The sequences of the specific primers used for the amplification of target genes are provided in Table 8. The template used is the pCR8/GW/topo vector containing any of target sequences. The primers are used in a PCR reaction with the following conditions: 5 minutes at 98° C., followed by 30 cycles of 10 seconds at 98° C., 30 seconds at 55° C. and 2 minutes at 72° C., followed by 10 minutes at 72° C. The resulting PCR fragment is analyzed on agarose gel, purified (QIAquick Gel Extraction kit, Cat. Nr. 28706, Qiagen), blunt-end cloned into Srf I-linearized pGNA49A vector (reference to WO00188121A1), and sequenced. The sequence of the resulting PCR product corresponds to the respective sequence as given in Table 8. The recombinant vector harbouring this sequence is named pGBNJ00XX.

E. Expression and Production of a Double-Stranded RNA Target in Two Strains of *Escherichia coli*: (1) AB309-105, and, (2) BL21(DE3)

[0342] The procedures described below are followed in order to express suitable levels of insect-active double-stranded RNA of insect target in bacteria. An RNaseIII-deficient strain, AB309-105, is used in comparison to wild-type RNaseIII-containing bacteria, BL21(DE3).

Transformation of AB309-105 and BL21(DE3)

[0343] Three hundred ng of the plasmid are added to and gently mixed in a 50 μl aliquot of ice-chilled chemically competent *E. coli* strain AB309-105 or BL21(DE3). The cells are incubated on ice for 20 minutes before subjecting them to a heat shock treatment of 37° C. for 5 minutes, after which the cells are placed back on ice for a further 5 minutes. Four hundred and fifty μl of room temperature SOC medium is added to the cells and the suspension incubated on a shaker (250 rpm) at 37° C. for 1 hour. One hundred μl of the bacterial cell suspension is transferred to a 500 ml conical flask containing 150 ml of liquid Luria-Bertani (LB) broth supplemented with 100 μg/ml carbenicillin antibiotic. The culture is incubated on an Innova 4430 shaker (250 rpm) at 37° C. overnight (16 to 18 hours).

Chemical Induction of Double-Stranded RNA Expression in AB309-105 and BL21(DE3)

[0344] Expression of double-stranded RNA from the recombinant vector, pGBNJ003, in the bacterial strain AB309-105 or BL21(DE3) is made possible since all the genetic components for controlled expression are present. In the presence of the chemical inducer isopropylthiogalactoside, or IPTG, the T7 polymerase will drive the transcription of the target sequence in both antisense and sense directions since these are flanked by oppositely oriented T7 promoters.

[0345] The optical density at 600 nm of the overnight bacterial culture is measured using an appropriate spectrophotometer and adjusted to a value of 1 by the addition of fresh LB broth. Fifty ml of this culture is transferred to a 50 ml Falcon tube and the culture then centrifuged at 3000 g at 15° C. for 10 minutes. The supernatant is removed and the bacterial pellet resuspended in 50 ml of fresh S complete medium (SNC medium plus 5 μg/ml cholesterol) supplemented with 100 μg/ml carbenicillin and 1 mM IPTG. The bacteria are induced for 2 to 4 hours at room temperature.

Heat Treatment of Bacteria

[0346] Bacteria are killed by heat treatment in order to minimise the risk of contamination of the artificial diet in the test plates. However, heat treatment of bacteria expressing double-stranded RNA is not a prerequisite for inducing toxicity towards the insects due to RNA interference. The induced bacterial culture is centrifuged at 3000 g at room temperature for 10 minutes, the supernatant discarded and the